SUPPLEMENTARY MATERIAL

Chemical composition of Ivorian *Artabotrys insignis* leaf oil. Combined analysis including ^{13}C NMR, to quantify germacrene A and β-elemene.

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Abstract: The chemical composition of leaf essential oil from *Artabotrys insignis* Engler & Diels collected from Cote d'Ivoire was determined by GC(FID), GC-MS and ^{13}C NMR. The main compounds were β-elemene (66.8%) and germacrene A (17.1%). The true content of germacrene A/β-elemene was obtained by combining GC(FID) and ^{13}C NMR data.
Keywords: *Artabotrys insignis*, Annonaceae, Côte d'Ivoire, essential oil, ^{13}C NMR.

Experimental

Plant material

A. *insignis* leaves were harvested in the forest of Petit Yapo near Agboville (south-east of Côte d'Ivoire). Voucher specimen LAA 12781 was deposited at the Herbarium of the National Floristic Center (University Felix Houphouët Boigny-Abidjan, Ivory Coast)

Essential oil isolation

Essential oil (EO) sample, was obtained by hydrodistillation from the fresh leaves with a Clevenger-type apparatus for a period of 3.5 h. The essential oil, light yellow coloured was dried over anhydrous sodium sulphate (Na_2SO_4), and then stored in a freezer until analyses. The yield, calculated on the fresh weight basis (w/w), was 0.33%.

Gas chromatography (GC)

Essential oil sample analysis was performed on Perkin-Elmer Clarus 500 gas chromatograph (FID) equipped two fused silica capillary columns (50 m x 0.22 mm, 0.25 μm film thickness) BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed as follow: 60-220°C at 2°C/min and then held isothermal at 220°C for 20 min. The carrier gas was Hydrogen, at a flow of 1.0 mL/min; injector port and detector temperature were 250°C. Sample was injected by splitting and the split ratio was 1:60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalisation, Retention indices (RI) were determined relative to the retention times of a series of n-alkanes (C7-C28) with linear interpolation ("Target Compounds" software from Perkin-Elmer).

Mass spectroscopy (GC-MS)

The essential oils were analysed with a Perkin-Elmer Turbo Mass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem, equipped with two fused silica capillary columns (50 m x 0.22 mm x 0.25 μm film thickness), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). Carrier gas: helium (0.8 mL/min); split, 1/60; injection volume: 0.5 μL; injector temperature: 250°C; oven temperature programmed from 60°C to 220°C at 2°C/min and then held isothermal (20 min); ion source temperature: 250°C; energy ionization: 70 eV; electron ionization mass spectra were acquired over the mass range 40-400 Da.

¹³C Nuclear magnetic resonance (¹³C-NMR)

^{13}C-NMR analysis was performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.623 MHz for ^{13}C, equipped with a 5 mm probe, in deuterated chloroform ($CDCl_3$), with all shifts referred to internal tetramethylsilane (TMS). ^{13}C NMR spectra were recorded with the following parameters: pulse width (PW), 4 µs (flip angle 45°); acquisition time, 2.73 s for 128 K data table with a spectral width (SW) of 22 000 Hz (220 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt. The number of accumulated scans was 3000 (50 mg of the mixture in 0.5 mL of $CDCl_3$). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier transformation.

Identification of components

Identification of individual components was based: (a) on comparison of their GC retention indices (RI) on polar and apolar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation (Target Compounds software of Perkin-Elmer), with those of authentic compounds (b) on computer search using digital libraries of mass spectral data and comparison with published data (National Institute of Standards and Technology 1999, König et al. 2001, Adams 2007) (c) on comparison of the signals in the ^{13}C NMR spectra of essential oils and the two fractions of chromatography with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi and Casanova 2006).

References

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